

Grundpraktikum Mikrobiologie, 4. Sem. (B.Sc.)
Universität Bremen

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Theory and Measurement of Bacterial Growth

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(04 May, 2007 / corrected version: 05 June, 2010)

A. Basic and practical aspects

1. Introduction

Life is essentially connected with growth. The phenomenon of growth can be studied scientifically from two different points of view:

(a) On the level of the **cell** or the **organism**: Here, the finely tuned cellular (metabolic and regulatory) processes underlying the increase in length and volume, division and replication are of interest. These processes can be studied without looking at the population as a whole.

(b) On the **population** level: Here the increase of the number of individuals or the population size and its mathematical description and modeling are of interest. Strictly speaking, this is the study of propagation (German: Vermehrung), but nevertheless the term growth is commonly used. This can be studied and modeled without knowledge of the cellular mechanisms.

A basic approach in microbiology is the study of growth from viewpoint (b). This is experimentally usually simple because growth (propagation) of bacteria can be very fast (in comparison to higher organisms) and requires only small culture volumes (flasks). The formulas for theoretical treatment are not really difficult.

2. Simple development of a growth equation

The following approach to develop the growth equation is not the proper mathematical one, but it is illustrative and easy to follow without having to understand differential equations.

Imagine a single cell of a bacterial species in a medium with all nutrients, oxygen, optimal temperature and pH. Assume that cells of this particular species double (divide) under optimal conditions every 3 h (Fig. 1). We thus write for the doubling time (also: generation time), t_d :

$$t_d = 3 \text{ h.}$$

To describe the increase of the number of cells over time (growth curve), we have to find an equation that expresses the cell number as a function (f) of time:

$$\text{cell number} = f(\text{time}),$$

meaning that **one variable is time** (independent variable) and **the other variable is the cell number** (dependent variable).

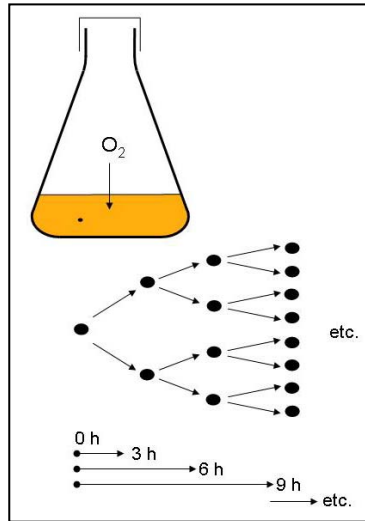


Fig 1. How to imagine cell growth that began with a single cell.

The following table presents how cell number and time correlate:

0 h	3 h	6 h	9 h	The time passed since the beginning of the experiment. For convenience, we here use multiples of the doubling time. Generally: t
1 cell	2 cells	4 cells	8 cells	The number of cells, after each division event (= appearance of a new generation). Generally: N
$= 2^0$ cell	$= 2^1$ cells	$= 2^2$ cells	$= 2^3$ cells	The same numbers of cells as above, but written in a manner that reveals the mathematical principle behind the increase of N . Generally: $N = 2^n$

However, the recognized equation

$$N = 2^n \quad (1)$$

does not yet include the other variable, t , for the growth curve. But what really is n ? It can be regarded as the number of division events or the ordinal number of the “daughter” generation. At the beginning, there has not been any division and there is no daughter generation, i.e. $n = 0$; after 3 h, there has been one division event and the 1st daughter generation has appeared, i.e. $n = 1$; etc. Because the number of division events increases with time, they can be expressed as a function of time: Just ask, how often the doubling time, t_d , is contained in (“fits into”) the time t passed since the beginning:

$$n = \frac{t}{t_d} \quad (2)$$

Now, the growth that began with **1 cell** can be described as:

$$N = 2^{t/t_d} \quad (3)$$

If growth would begin with **5 cells** (imagine the same flask with 5 independently dividing cells, or 5 separate flasks each with 1 cell), the equation would be

$$N = 5 \cdot 2^{t/t_d} \quad (4)$$

If growth would begin with **any cell number, N_0** , the equation would be

$$N = N_0 \cdot 2^{t/t_d} \quad (5)$$

Note that the cell number in a culture will not increase stepwise in a “stair function” ($N_0 \rightarrow 2 N_0 \rightarrow 4 N_0 \rightarrow 8 N_0$ etc.), but steadily. This is because cells do not divide synchronously; some will divide earlier, some later. Only with special experimental tricks it is possible to obtain synchronous, stepwise propagation over a certain period of time.

Some like writing equation (5) without a denominator in the exponent and replace the reciprocal doubling time, $1/t_d$, by another constant, the doubling rate (also: division rate, generation rate), ν , the number of divisions (generations) per unit time:

$$\frac{1}{t_d} = \nu \quad (6)$$

and thus obtain

$$N = N_0 \cdot 2^{\nu t} \quad (7)$$

Like (5), also this is sufficient to describe growth. Still, expression of exponential functions with the basis 2 is not common in natural sciences. Much more common are exponential functions with the basis e (Euler number, $e = 2.71828\dots$, a mathematically important non-periodic number), so-called e -functions. Mathematically, an e -function is the genuine, natural or “The exponential function”. Because a given exponential function can be expressed with any basis, we can also express equation (5) as an e -function without changing its curvature. By definition of the natural logarithm, the number 2 can be written as

$$2 = e^{\ln 2} \quad (8)$$

This converts equation (5) to

$$N = N_0 (e^{\ln 2})^{t/t_d} = e^{(\ln 2 / t_d) t} \quad (9)$$

(with $\ln 2 = 0.6931\dots$). For a given species of a bacterium, the doubling time, t_d , is a kind of species-specific, unchangeable characteristic: every bacterial species has a particular, genetically fixed doubling time under optimal growth conditions. The doubling time of a given bacterial species growing optimally can thus be regarded as a fixed value. Hence, we can define a new constant, μ :

$$\boxed{\frac{\ln 2}{t_d} = \frac{0.693}{t_d} = \mu} \quad (10)$$

The new constant μ is termed the **specific growth rate** or often simply **growth rate**, (unit: d^{-1} , h^{-1} , or min^{-1}). With this, we obtain the growth equation in its well-known form:

$$\boxed{N = N_0 e^{\mu t}} \quad (11)$$

Equations (6) and (10) reveal the connection

$$\mu = (\ln 2) \nu = 0.693 \nu \quad (12)$$

In former times, ν and equations (6), (7) and (12) were given (too) much consideration in the theory of bacterial growth. Today they represent only a side aspect (sometimes even

leading to confusion), and sound evaluation of growth experiments is possible without using v . Equations (10) and (11) are definitely the really important ones.

The theoretical approach explained here is not quite “clean” from a mathematical point of view, because of the discontinuous states (1, 2, 4, 8 etc.). Those who are interested in the mathematical “clean” approach can go through section B.

3. From cell counting to optical measurement

For the measurement and plot of a growth curve (N versus t), we would have to determine the cell number in a culture at the beginning and then after different time intervals. Because we cannot count all cells N in a culture volume, V , we have to count them in a representative sample (aliquot) with the volume V^{sample} taken from the culture, for instance in a microliter placed in a microscopic counting chamber. The revealed cell number, N^{sample} , is only a tiny proportion of the total cell number, N , just as V^{sample} is a tiny proportion of the total cell number. So it is almost immediately intuitive that the ratio $N^{\text{sample}}/V^{\text{sample}} = N/V$. This ratio, the cell number per volume unit (cells ml^{-1} , or cells l^{-1}), is a decisive parameter also termed cell density or “cell concentration”:

$$\frac{N^{\text{sample}}}{V^{\text{sample}}} = \text{Cell density} \quad (13)$$

Hence, the cell number in equation (11) can be referred to the culture volume, V . The equation then expresses the increase in cell density:

$$\frac{N}{V} = \frac{N_0}{V} e^{\mu t} \quad (14)$$

which is also

$$\frac{N^{\text{sample}}}{V^{\text{sample}}} = \frac{N_0^{\text{sample}}}{V^{\text{sample}}} e^{\mu t} \quad (14a)$$

This opens a possibility to avoid the time-consuming and “boring” cell counting, because the cell density can be measured optically in a photometer. If a culture is placed in a cuvette, a light beam passing through the cuvette will be scattered more or less by the cells, depending on the cell density; to our eye this appears as turbidity (see section 4). Actually, the scattering intensity or turbidity, often termed optical density (OD), is proportional (\sim) to the cell density:

$$\frac{N^{\text{sample}}}{V^{\text{sample}}} \sim OD \quad (15)$$

We can determine a proportionality factor or conversion factor, a , and get an equation. This factor, once determined, allows calculating the cell density from any measured OD :

$$\frac{N^{\text{sample}}}{V^{\text{sample}}} = a \cdot OD \quad (16)$$

However, for measurement of a growth curve, we do not even have to determine such a conversion factor because it disappears if we insert $a \cdot OD$ and correspondingly $a \cdot OD_0$ (OD_0 = optical density at $t = 0$) in the above equation:

$$a \cdot OD = a \cdot OD_0 e^{\mu t} \quad (17)$$

$$OD = OD_0 e^{\mu t} \quad (18)$$

We can thus simply follow the OD by optical measurement and plot it versus t to obtain a growth curve; from this we can calculate μ of the bacterium. For data evaluation, we can display the exponential curve as a straight line if we plot the OD on a logarithmic scale semilogarithmically versus time on a linear scale (semilogarithmic display; Fig. 2).

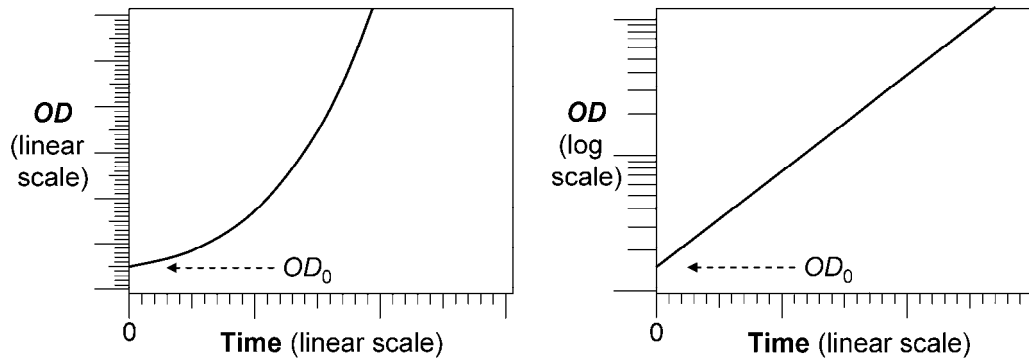


Fig. 2. Linear and semilogarithmic display of exponential growth. Be aware of zero points: There is a zero-point on the time scale. However, there is no zero-point on a log scale. Also, growth can never start at $OD=0$ (which would mean that there are no cells to begin with).

4. Practical considerations

The OD is measured in a (spectro)photometer (Fig. 3). The underlying principle is that most of the light scattered by the cells no longer reaches the photoelectric cell, so that the electric signal is weaker than with a cell-free cuvette. The OD of a bacterial culture is thus primarily **not an absorbance**, as in the case of a dissolved dye. Cells of many bacteria are almost colorless and real light absorption is marginal. It is therefore not correct (even though unfortunately common) to designate the OD of a culture an absorption; the most appropriate term would indeed be **turbidity**. Only strongly pigmented cells such as phototrophic (purple and green) bacteria cause a significant absorption of light in addition to scattering. One should be aware that the photoelectric cell does not “know” what causes the decrease in light intensity. It just notices “less light” which is displayed as a number, usually as extinction value. But even if the display has the label “absorption”, you know that you are dealing with turbidity.

The practical use of the photometer requires consideration of four points:

(a) The intensity of light scattering and thus the OD of a culture depend on the wavelength. Scattering of short wavelengths (such as of blue light) is stronger than that of long wavelengths (such as of red light). You can choose any wavelength for measurement, but once you have chosen you must **keep this wavelength** for all following measurements. This wavelength (e.g. 600 nm) should be indicated as subscript (e.g. OD_{600}).

(b) The OD measured by a photometer depends on the geometry of the light beam, the position of the cuvette, and the photoelectric cell, i.e. on the photometer model and manufacturer. Hence, you cannot change the model of the photometer within an experiment. One should not even change between two photometers of the same model because there can be slight differences.

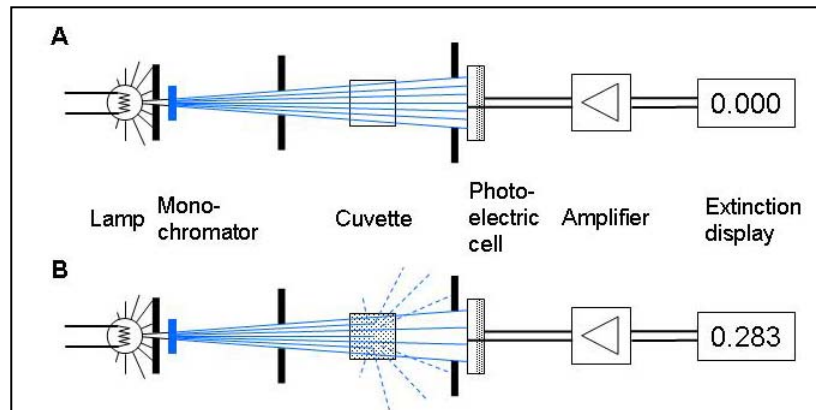


Fig. 3. Measurement of the *OD* of a culture. **A** If there is clear sterile medium in the cuvette, the intensity of the light that reaches the photoelectric cell is taken as the reference intensity and adjusted to zero extinction or zero *OD*. **B** With bacterial cells in the cuvette, a significant portion the light is scattered and no longer reaches the photoelectric cell. The weaker electric signal is converted to an extinction or *OD* value.

(c) Because you want to measure only the *OD* caused by the cells, any light absorption caused by the medium (brownish yeast extract, etc.) has to be subtracted. This is most simply done if you initially fill the cuvette with sterile (clear) growth medium, place it in the photometer and adjust the displayed extinction value to 0.000. Every subsequently measured value above this is caused by the cells. Verify from time to time again with sterile medium that the zero-point has not drifted; readjust if necessary. Also, mark your cuvette and place it always in the same orientation (don't turn it the next time by 180°). Empty, rinse and dry (as far as possible) the cuvette for the next measurement. Don't touch the clear ("window") sides of the cuvette. Fingerprints must be removed.

(d) The proportionality between *OD* and cell density exists only for $OD \leq 0.4$ (approximately). Because higher *OD* values are no longer proportional to the cell density (Fig. 4), their direct plot versus t yields a wrong (distorted) growth curve in the upper range. If a culture approaches such a critical *OD*, samples must be diluted prior to measurement by a defined dilution factor, d . The measured value is then divided by d to get the corrected or calculated optical density, OD_{corr} .

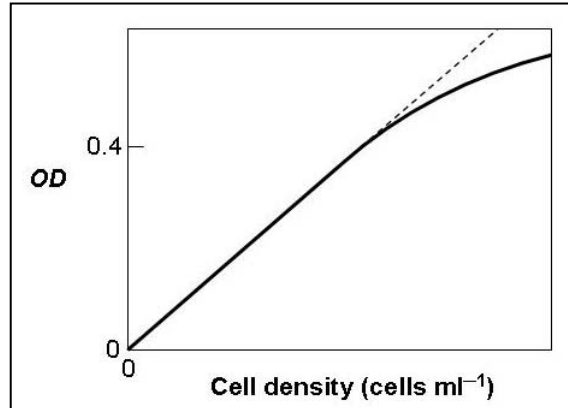


Fig. 4. The OD above a certain critical value (for many bacteria around 0.4) deviates from proportionality to the cell density.

Examples:

Below critical OD:

Measured: OD = 0.270

Sample diluted by $d = \frac{1}{2}$ (1 ml sample + 1 ml sterile medium): OD = 0.135.

Division by d : $OD_{\text{corr}} = 0.135 : \frac{1}{2} = 0.270$ (as above; dilution would not have been necessary)

Above critical OD:

Measured: OD = 0.540

Sample diluted by $d = \frac{1}{2}$ (1 ml sample + 1 ml sterile medium): OD = 0.288.

Division by d : $OD_{\text{corr}} = 0.288 : \frac{1}{2} = 0.576$ (higher value; dilution was necessary to reveal this).

Hence, the table for measurement of a growth curve may look as follows:

Time of the day	Time, t (min)	OD undiluted	Dilution factor, d	OD diluted	OD_{corr}
08:15	0	0.055	(no dilution \rightarrow) 1	-	0.055
etc.	etc.	etc.	etc.	etc.	etc.
12:00	225	0.685	(1 + 3 ml \rightarrow)* $\frac{1}{4}$	0.206	0.824
etc.	etc.	etc.**	etc.	etc.	etc.**

* The resulting total volume is 1 + 3 = 4 ml. Hence, the cells from originally 1 ml are now in 4 ml, the 4-fold volume. The dilution factor is thus $\frac{1}{4}$.

** If OD and/or OD_{calc} are already plotted while the experiment is going on, the curve or line may give you a “feel” for its further development. With such “feel”, you can guess the forthcoming value and think of a useful dilution factor, without having to go through “trial and error” for each sampling point.

In reality, the semilogarithmic plot of OD_{calc} versus t does just yield just the theoretically expected straight line, but a more complicated growth curve. The reason for this is that cells inoculated into new medium do not immediately start growing with their optimal μ (the value you are interested in). They must first restart (“wake up”) their machinery for the synthesis of ATP, DNA, RNA, protein, etc., which had been turned down during their previous storage (in the refrigerator for instance). On the other hand, a culture cannot grow forever, because there is only a limited amount of nutrient and a limited culture volume. (You may calculate the time needed until the aforementioned cells with an

assumed occupational volume of $1 \mu\text{m}^3$ would completely fill a swimming pool.) Actually, we can distinguish between different phases of a culture (Fig. 5). For calculation of μ , only the exponential phase is of interest; this is obvious from the linear slope (linear increase) in the semilogarithmic plot.

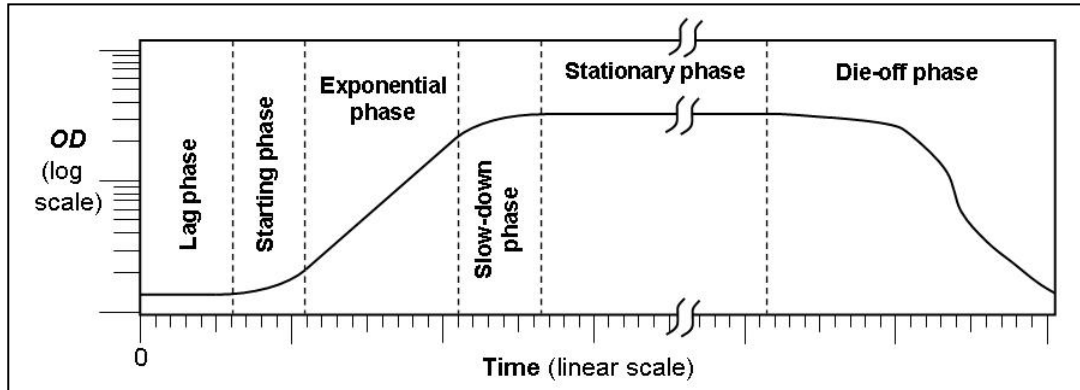


Fig. 5. Phases in a bacterial culture. (Common German expressions: Lag-Phase, Anlauf-Phase, exponentielle Phase, Verlangsamungsphase, stationäre Phase, Absterbephase.) The stationary phase often lasts much longer than all the preceding phases together. The die-off phase can be quite unpredictable and irregular. Growth experiments in a laboratory course usually get only to the slow-down phase or at best to the beginning of the stationary phase. But what counts is the exponential phase. There are theoretical approaches to describe also the slow-down phase, but this is not part of a basic growth experiment.

Remarks to avoid a common misconception of the stationary phase: The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids. An awkward but unfortunately wide-spread explanation is that the stationary phase results from a situation in which growth rate and death rate have the same values (newly formed cells per time = dying cells per time); but this is not logic, and it is better to forget this. Such an explanation would not be in accordance with the observed substrate depletion and also could never explain the rather “smooth”, horizontal linear part of the curve during the stationary phase. Death of cells as a function of time is rather unpredictable and very difficult to explain. Another not really logic explanation of the stationary phase is that “there isn’t anymore enough space for the cells”. However, under the microscope you will see that there is still plenty of water between the cells. Only in an agar colony with densely packed cells space is obviously limiting.

The exponential phase is used to determine μ from two data points, OD_1 and OD_2 that you chose exactly **on** the fitted line. Do not use just two measured values (which mathematically are never exactly on the best-fitted line). If you would do so, you would not make use of the best-fitted line. There are two possibilities:

(a) An easy graphic way is to chose the two OD -values such that OD_2 is $2 \cdot OD_1$. Then the time distance directly equals t_d (Fig. 6). From this, μ can be calculated.

(b) The mathematical approach is to chose any OD_2 and OD_1 and the corresponding t_2 and t_1 values, again from the line. Then equation (36a) is used to calculate μ . From this, t_d can be calculated.

Always indicate t_d and μ with the correct units!

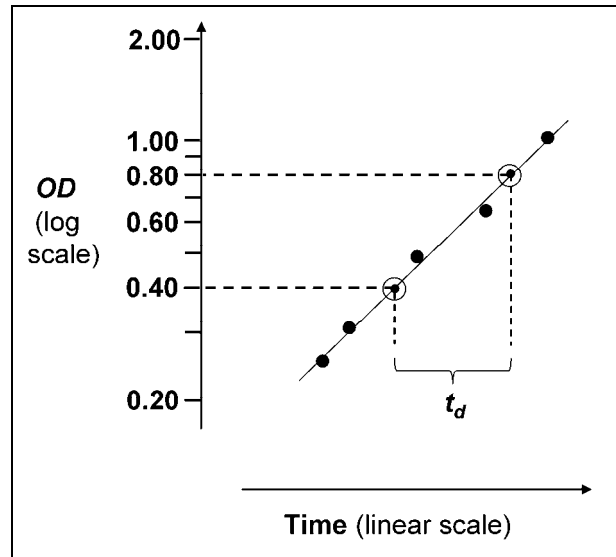


Fig. 6. Simple graphic determination of t_d . Symbols: ●, measured points; ⊙, points chosen on the line (“mathematical points”).

B. Mathematical development of the growth equation

The principle of this approach is very basic in natural sciences

The increase in cell numbers, ΔN (= number of newly produced cells) during a given time interval, Δt , is proportional (\sim) to the actual number of cells, N . (If a population of 20,000 cells produces 1200 new cells in 1 h, a 4-fold bigger population of 80,000 cells of the same type of microorganism will produce 4 times as many, viz. 4800, new cells in 1 h):

$$\frac{\Delta N}{\Delta t} \sim N \quad (19)$$

Because the newly produced cells always add to the population, i.e. N increases steadily, we have to regard time intervals (and accordingly numbers of newly formed cells) that are as small as possible. Mathematically we thus have to deal with infinitesimal increases or differentials:

$$\frac{dN}{dt} \sim N \quad (20)$$

To get from proportionality to an equation, a proportionality factor, μ , is introduced. This is the specific growth rate or often termed simply growth rate.

Remark: Strictly speaking, dN/dt is the “absolute” growth rate (number of cells formed per time), and μ the “specific” growth rate, viz. the “absolute” growth rate related to the population size, N (number cells formed per time and existing number of cells).

$$\frac{dN}{dt} = \mu N \quad (21)$$

Sorting of variables yields:

$$\frac{1}{N} dN = \mu dt \quad (22)$$

This equation contains variables in the form of differentials and therefore represents a **differential equation**. This has to be solved to get away from differentials. From many “daily” equations we are used to expect a number as the solution. In contrast, the solution of a differential equation is an **equation** (with variables that are no longer differentials). There isn’t any standard procedure to solve differential equations, and often they are tricky. But solution always has to go through integration. With the above, “sorted” differential equation (note that μ is a constant), integration can be performed directly:

$$\int \frac{1}{N} dN = \mu \int dt \quad (23)$$

Nevertheless, the common rule of integration does not work in this case, because integration of the term $1/N$ or N^{-1} would yield a “result” with the nonsense-expression $1/0$. However, it is known that the first derivative of $y = \ln x + \text{constant}$ (for any *constant*) is $y' = 1/x$ (see books of basic mathematics). Hence, integration (the reversal operation of forming the first derivative) yields

$$\ln N = \mu \cdot t + \text{constant} \quad (24)$$

The undefined integration *constant* can be fixed if we define the initial conditions: At the beginning, namely $t = 0$, the cell number is $N_{t=0}$ or simply N_0

$$\ln N_0 = \mu \cdot 0 + \text{constant} \quad (25)$$

$$\text{constant} = \ln N_0 \quad (25a)$$

With this, equation (24) becomes a defined one:

$$\ln N = \mu t + \ln N_0 \quad (26)$$

$$\ln N - \ln N_0 = \mu t \quad (27)$$

$$\ln \frac{N}{N_0} = \mu t \quad (28)$$

$$\frac{N}{N_0} = e^{\mu t} \quad (29)$$

$$\boxed{N = N_0 e^{\mu t}} \quad (30)$$

Remark: Now the theoretical approach is mathematically appropriate, but the formula cannot be taken “100% serious” for describing the increase in cell numbers. Mathematically, the exponent μt is allowed to be any real number ($\mu t \in \mathbb{R}$) that can yield any positive real number for N (already simply $\mu t = 1$ yields $N = N_0 \cdot 2.71828\dots$). In the world of organisms, of course, N can be only a positive integer ($N \in \mathbb{N}$). If a starting population of $N_0 = 5$ cells grows with $t_d = 2$ h, the formula predicts $N = 14.142\dots$ cells after 3 h. Yet we know that there are still 10 cells, like after 2 h. The initial approach was obviously not so bad. But the inconsistency disappears if we look at the cell mass, X , (rather than at cell numbers) in a culture. This indeed increases continuously (if we forget that the increments are now molecules) according to $X = X_0 e^{\mu t}$, where X_0 is the cell mass at $t = 0$. This necessarily implies that the mass of a single cell is not constant: After a division event, the daughter cell has its lowest mass; this then increases (usually cell length increases) until the next division occurs.

There are two further important connections:

(a) From equation (28), we get the same expression for μ as in equation (10), but in a more intelligible manner: If the time that has passed is exactly the doubling time, we can write $t = t_d$; within this time span, N_0 has increased to $2 N_0$, such that equation (28) yields

$$\ln \frac{2 N_0}{N_0} = \mu t_d \quad (31)$$

and hence again

$$\boxed{\mu = \frac{\ln 2}{t_d}} \quad (10)$$

(b) Equation (27) allows calculation of μ from any two points that we place on the plotted growth curve. According to relationship (15), equation (27) can be also expressed as

$$\ln OD - \ln OD_0 = \mu t \quad (32)$$

For two OD values chosen on the line, OD_1 and OD_2 , the corresponding time points are t_1 and t_2 , respectively:

$$\ln OD_1 - \ln OD_0 = \mu t_1 \quad (33)$$

$$\ln OD_2 - \ln OD_0 = \mu t_2 \quad (34)$$

The difference (34) – (33) yields

$$\ln OD_2 - \ln OD_1 = \mu (t_2 - t_1) \quad (35)$$

and for μ

$$\mu = \frac{\ln OD_2 - \ln OD_1}{(t_2 - t_1)}, \quad (36)$$

or with conversion to the decadic logarithm, \lg

$$\boxed{\mu = \frac{2.303 (\lg OD_2 - \lg OD_1)}{(t_2 - t_1)}} \quad (36a)$$

Note that application of this formula absolutely needs the \lg of the two OD data points. If OD has been directly plotted on a logarithmic scale, the values are not yet the logarithms; only their graphic display is logarithmic. So you still have to form the logarithms.

Typos? Remarks? Better suggestions? Please send a message to fwiddel[at]mpi-bremen.de (in German or English)